## Remarks

The undersigned thanks the Examiner for granting a telephonic interview on December 21, 2004, and for his helpful suggestions at that time. The following Amendment incorporates changes discussed during the interview. In particular, the claims have been amended to recite phenotypic characteristics of the claimed cells and mice.

Please note that the references to pages and line numbers herein refer to the amended specification that was submitted on October 15, 2001, rather than the specification that was originally filed.

The claimed invention

The claims as amended are drawn to mice and mouse cells whose genome is heterozygous or homozygous for a mutation engineered into the Erk5 gene or chimeric mice containing cells that are heterozygous for the mutation. In a homozygous state the mutation results in a functionally deficient Erk5 gene and embryonic death characterized by a lack of vasculature, revealing that Erk5 plays a role in angiogenesis. When interbred, heterozygous mice of the invention produce homozygous offspring that undergo embryonic death characterized by a lack of vasculature.

Rejections under 35 U.S.C. § 112

Claims 1-7 and 12 stand rejected under 35 U.S.C. § 112, first paragraph, on the ground that the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention.

With respect to claims to mice or cells homozygous for a mutation in the Erk 5 gene (claims 3 and 4), the Examiner has indicated that such claims are enabled wherein the mutation results in a non-functional Erk5 gene, wherein a transgenic mouse embryo homozygous for such a mutation does not produce a functional Erk5 protein, and wherein said mouse embryo is characterized by a lack of vasculogenesis and angiogenesis. Claim 3 has accordingly been amended to indicate that the mutation results in failure to produce a functional Erk5 protein. Support for this amendment is found in original claim 3, since a non-functional Erk5 gene would

inherently result in failure to produce a functional Erk5 protein. As indicated in the specification at p. 9, lines 10-14, confirmation that the genetically altered embryo is defective in Erk5 production can be achieved by analysis of the embryo's expressed proteins for the absence of molecules corresponding to Erk5. Such methods of analysis, e.g., immunoblots, are well known in the art. Applicants submit that claim 3, as amended, is allowable and that claim 4, which depends on claim 3, is also allowable. Withdrawal of the rejection is respectfully requested.

With respect to the remaining claims, the Examiner contends that the specification does not teach how to use a heterozygous mouse or cells because since "there is no difference in the phenotype of the heterozygous mouse or the cells therefrom, an artisan would not be able to differentiate a wild type mouse from a heterozygous knockout mouse...and would therefore not know how to use the mouse." Applicants respectfully disagree. While the differences in the phenotype of wild type and heterozygous mice or cells that are described in the specification are not discernable from visual observation of the gross physical appearance of the mice or cells, the specification does describe a number of differences that the skilled artisan could readily use to distinguish between wild type and heterozygous mice or cells and describes methods of using these differences to make the distinction. For example, p. 6, line 33 – p. 7, line 17, describes generating cells that are heterozygous for a non-functional Erk5 gene. These cells are created by transforming a cell with a construct containing genomic DNA in which a part of the DNA encoding Erk5 is replaced with a marker gene and wherein the construct preferably contains a second, different marker gene, such as tk. Following transformation, "Cells containing this mutated Erk5 gene are identified by growth in media selective for one or both of the marker genes." (p. 7, lines 15-17)

Applicants used the double selection process to generate cells in which homologous recombination occurred and then identified such cells. As described in the specification at p. 7, lines 20-25, "homologous recombination is confirmed by Southern blotting against DNA isolated from the transformed cells, using a probe specific for the Erk5 gene." One of ordinary skill in the art would readily recognize that such a probe would detect a single band corresponding to Erk5 in DNA isolated from wild type cells. One of ordinary skill in the art would also recognize that such a probe would either detect no Erk5 band or would detect a single band of a different size from that detected in wild type cells in DNA isolated from cell homozygous for an Erk5 mutation. However, such a probe would detect two bands of different sizes in DNA isolated

from cells heterozygous for the Erk5 mutation. One band would correspond to the wild type allele while the other band would correspond to the mutated allele.

The specification describes a particular example of generating and identifying heterozygous cells at p. 21, line 7 – p. 22, line 3, which states that following transformation of cells with a construct (targeting vector), genomic DNA was isolated from colonies of cells originating from transformants that survived drug selection and was then subjected to restriction digestion and Southern blotting with a probe isolated from the genomic Erk5 clone. As stated at p. 22, lines 1-3, "Either a 10 kB wild-type band or a 6 kB mutant band would be detected by the probe." Clearly, only the wild type band would be detected in wild type cells, and only the mutant band would be detected in homozygous mutant cells. In cells that are heterozygous for the mutation, both bands would be detected. Applicants therefore submit that one of ordinary skill in the art would readily be able to distinguish heterozygous cells from wild type cells. Such methods would be equally applicable to heterozygous cells generated by homologous recombination *in vitro* or to cells isolated from heterozygous or chimeric mice. Furthermore, in the case of cells that are made by homologous recombination as described in the specification, or cells that are isolated from either a chimeric mouse or a heterozygous mouse made as described in the specification, one of ordinary skill in the art would be able to distinguish heterozygous cells from wild type cells because the heterozygous cells are able to grow in media that are selective for the drug resistance gene(s) that was used to create the Erk5 mutation (see p. 8, lines 1-10). One of ordinary skill in the art would typically be aware of the method by which the cells were made (e.g., by introducing a targeting construct containing particular drug resistance genes into the cells) and would have this information available when distinguishing the cells.

Breeding of heterozygous mice would be expected to result in mice that are either (i) homozygous wild type (+/+); (ii) heterozygous for the mutation (+/-), or homozygous for the mutation (-/-). Applicants determined the genotype of adult mice generated from breeding of heterozygous mice. As shown in Table 1 (p. 22), Applicants were able to identify heterozygous mice and to distinguish them from wild type mice. Applicants thus submit that one of ordinary skill in the art would readily be able to distinguish heterozygous mice or chimeric mice from wild type mice (e.g., by performing a Southern blot on blood cells from the mice and detecting two bands in DNA isolated from cells obtained from either heterozygous or chimeric mice). Other techniques for detecting specific DNA bands, such as PCR using appropriate primers,

would also be readily evident to one of ordinary skill in the art. Alternately, the relevant portions of DNA could be sequenced.

One of ordinary skill in the art would also be aware of other methods that could be used to distinguish either heterozygous cells or mice from wild type, e.g., by analyzing Erk5 mRNA and/or protein structure and/or levels in the cells or mice. Suitable methods known to one of ordinary skill in the art include Northern blots, reverse transcription PCR (RT-PCR), including quantitative PCR, single cell PCR, immunoblots, kinase assays, etc.

In summary, Applicants submit that the specification does teach one of ordinary skill in the art how to differentiate wild type cells from heterozygous cells and how to distinguish wild type mice from heterozygous mice and clearly teaches and exemplifies how to use heterozygous mouse ES cells to generate chimeric mice which can then be bred to produce heterozygous mice, i.e., by identifying recombinant cells having a mutation in the Erk5 gene using the methods described above and injecting the cells into a blastocyst. (See p. 7, lines 26-34, which describes the technique in general terms, and p.21, line 21 – p. 22, line 7, describing specifically the work performed by Applicants). The specification further teaches and exemplifies how to use the chimeric mice to generate heterozygous mice and how to use the heterozygous mice to generate homozygous mutant embroys such as those of claim 3, i.e., by breeding the mice (see p.7, lines 26-30, and p. 22, lines 26-32), describing the technique in general, and p. 22, lines 3-7 and 11-14, describing work performed by Applicants). Thus Applicants submit that the specification does teach how to use heterozygous cells or mice and contains working examples of such use.

The Examiner further states that since "protein produced from one allele of the gene is sufficient for supporting normal function in the heterozygous mice and therefore cells isolated from the heterozygous mice will not have any functional abnormality and therefore, an artisan will not know how to use these cells." Applicants respectfully disagree that cells isolated from the heterozygous mice will not have any functional abnormality. It is evident that heterozygous cells contain only one functional Erk5 gene and thus will not transcribe mRNA encoding a functional Erk5 protein from this gene. The cells are thus expected to produce less functional Erk5 protein than wild type cells. See p. 7, line 21-22, of the specification, which states that heterozygous cells are useful for studying the effect of *reduced expression of Erk5*. While such a reduced amount of Erk5 protein is sufficient to support life and does not seem to cause a phenotypic abnormality evident from visual observation, Applicants submit that the

heterozygous cells do have a detectable functional abnormality. As pointed out in the Office Action Response filed Dec. 1, 2003, the specification states that cultures of heterozygous cells are "useful to assay for compounds that potentially rescue the Erk5 mutation" (p. 7, lines 3-6,) and mentions methods of measuring Erk5 kinase activity (p. 11, lines 21-26). Applicants therefore submit that the specification teaches one of ordinary skill in the art how to use heterozygous cells to screen for compounds that rescue the Erk5 mutation. Such compounds could be identified based on their ability to cause an increase in the level of Erk5 kinase activity within the heterozygous cells, thereby restoring the Erk5 kinase activity to higher levels, e.g., levels closer to those in wild type cells. Alternately, compounds that cause an increase in Erk5 mRNA and/or protein expression could be identified by performing Northern blots, PCR, and/or immunoblots.

Furthermore, one of ordinary skill in the art would know how to use the heterozygous cells to screen for compounds that inhibit Erk5 activity, as taught at p. 13, lines 14-18. Such compounds would be identified using similar assays to those useful for identifying compounds that rescue the Erk5 mutation, e.g., assays that measure Erk5 mRNA levels, protein levels, and/or kinase activity in the heterozygous cells. Rather than identifying compounds that increase Erk5 mRNA levels, protein levels, and/or kinase activity, the assay would identify compounds that decrease such levels or activity. In the case of screening assays performed using heterozygous mice, inhibitors of Erk5 could be identified because sufficient inhibition of the Erk5 protein expressed from the non-mutated Erk5 allele would be expected to mimic the effect of a homozygous mutation in the Erk5 gene, i.e., it would result in embryonic lethality with a failure of vasculogenesis and angiogenesis in homozygous offspring of the heterozygous mice.

The Examiner also states that, "since these cells or mouse will behave like a normal mouse or cells...how would an artisan know that the effect of a compound in a screening assay is because of the effect of the compound on the mutated gene". Applicants submit that it is not necessary for the compound to act on the mutated gene for the screening assay to be useful or in order for the skilled artisan to know how to use the cells and mice in the screening assay. Applicants further submit that it is not necessary for the artisan to know what gene (or protein) the compound acts on in order for the screening assay to be useful or in order for the artisan to know how to use the cells and mice in the screening assay. As long as the compound does indeed rescue the Erk5 mutation the compound is of interest, regardless of whether it does so by

acting on the mutated gene. In fact, since the mutated gene is non-functional, the compound may more likely act on the non-mutated (wild type) allele of Erk5, e.g., by increasing or decreasing expression of Erk5 from that allele or by increasing or decreasing the kinase activity of Erk5 protein expressed from the wild type allele. A compound of interest may act by increasing or decreasing expression or functional activity of a second protein that normally activates existing Erk5 protein or substitutes for Erk5 protein within the cells. Furthermore, if the artisan wishes to confirm that a compound identified in the assay does indeed act specifically on Erk5, the artisan will be able to conduct appropriate control experiments, e.g., using wild type cells or mice. Designing and conducting control experiments is well known to those in the art.

In conclusion, Applicants submit that the specification teaches one of ordinary skill in the art how to use heterozygous cells and how to use heterozygous or chimeric mice and specifically exemplifies the use of heterozygous cells and mice. The specification teaches that heterozygous cells can be distinguished from wild type cells by Southern blot or by their ability to grow in selective media. These methods can also be applied to distinguish heterozygous or chimeric mice from wild type mice, e.g., by isolating cells from such mice and subjecting them to the foregoing tests. Furthermore, one of ordinary skill in the art would immediately recognize that numerous other assays based on differences in either the DNA, mRNA, or Erk5 protein in wild type cells or mice and in heterozygous cells, heterozygous mice, or chimeric mice could be used to make the distinction. The specification further teaches that heterozygous cells or mice are useful for performing assays to identify compounds that rescue an Erk5 mutation or inhibit Erk5. Such assays are well known in the art, and Applicants submit that implementing them for Erk5 is well within the capacity of the skilled artisan.

Claims 1-2, 5-7, and 12 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner states that the rejection is made for the same reasons given in the previous office action of 10/15/01. (Applicant believes that the Examiner intended to refer to the office action of July 30, 2003.) In particular, the Examiner asserts that the specification has disclosed the phenotype of a homozygous mouse embryo but does not describe the characteristics of a heterozygous mouse. The Examiner further notes that claims drawn to heterozygous or chimeric mammals (*sic*) recite characteristics that are produced in a homozygous mouse and not in a heterozygous mouse.

The written description requirement carries "a strong presumption that an adequate written description of the invention is present when the application is filed." See Fed. Reg., Vol. 66, No. 4, 1104 (Guidelines for the Examination of Patent Applications Under the 35 U.S.C. § 112, ¶ 1, "Written Description" Requirement) and also the Revised Interim Written Description Guidelines Training Manual, Synopsis of Written Description Guidelines at p. 3. Claims to heterozygous and chimeric mammals and cells were present in the application as filed, and amendments made to the claims to recite mice rather than mammals and to indicate the characteristics of the claimed cells, mice, and homozygous offspring thereof, are all clearly supported by the specification. The Examiner bears the burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the original disclosure a description of the invention defined by the claims (Revised Interim Written Description Guidelines Training Manual, p. 3). Applicants submit that the Examiner has not met this burden.

In the office action of July 30, 2003, the Examiner indicated that in the case of a transgenic animal, a determination of whether the written description requirement for description of a representative number of species is met involves determining "whether phenotypic consequences or other characteristics of the animals resulting from altering the genotype have been described". While not conceding that this interpretation is correct, Applicants submit that such phenotypic consequences or other characteristics of the heterozygous cells and the heterozygous or chimeric mice have been described. In the case of the instantly claimed heterozygous cells and heterozygous mice, each cell bears a mutation in one allele of Erk5, such that the mutated gene is incapable of producing a functional Erk5 protein. Applicants submit that such a mutation would result in reduced expression of Erk5, as indicated at p. 7, line 21-22, which states that heterozygous cells are useful for studying the effect of *reduced expression of Erk5*. Such decreased expression is readily observable, e.g., by methods mentioned above such as Northern blot, RT-PCR, immunoblot, etc.

Applicants submit that the written description requirement refers to the specification and does not require that the description be contained in the claims themselves. Nevertheless, claims 2, 5, and 7 have accordingly been amended to recite a characteristic of the cells, i.e., that the mutation results in a functionally deficient Erk5 gene and lack of expression of mRNA encoding functional Erk5 protein from the gene in the cells. In the case of chimeric mice, some cells will be wild type while others will be heterozygous. The presence of heterozygous cells is a

characteristic of the chimeric mice and can be determined as described above. In the case of the heterozygous mice, all of the cells will be heterozygous. The presence of heterozygous cells is a characteristic of the heterozygous mice and can be determined as described above.

The Examiner notes that the claims drawn to heterozygous or chimeric mammals (*sic*) recite characteristics that are found in the homozygous mice rather than in the heterozygous or chimeric mice. As described in the specification, the heterozygous mice will, when interbred, predictably give rise to homozygous embryos that have the phenotype recited in the claim. The characteristic of giving rise to such embryos is a characteristic of the heterozygous mice. As noted above, Applicants submit that the written description requirement does not require that the description be contained in the claims themselves. Nevertheless, claim 1 has been amended to indicate that interbreeding of the heterozygous mouse results in at least some homozygous embryos that fail to produce a functional Erk5 gene and undergo embryonic death characterized by a lack of vasculogenesis and angiogenesis. Similarly, the chimeric mice will, when interbred, predictably give rise to heterozygous mice that will, when interbred, predictably give rise to homozygous embryos as described in claim. Claim 6 has also been amended to recite that interbreeding of the chimeric mouse results in at least some offspring that are heterozygous for a mutation engineered into the Erk5 gene, wherein interbreeding of said heterozygous offspring results in at least some homozygous embryos that fail to produce a functional Erk5 gene and undergo embryonic death characterized by a lack of vasculogenesis and angiogenesis.

Applicants note that U.S. 5,650,550, which the Examiner cited in rejecting certain claims in the previous office action, includes claims to heterozygous and chimeric mice having mutations in an estrogen receptor gene. The claims recite characteristics that are found in the homozygous mice rather than in the heterozygous or chimeric mice (see claims 2 and 3). A review of the specification does not reveal any gross visible phenotype for the heterozygous or chimeric mice. The specification states that PCR amplification was used to distinguish heterozygous from homozygous mice. This method is standard in the art, as noted above. The specification further states that with respect to estrogen responsiveness (a lack thereof being a phenotypic characteristic of the homozygous mice), "The wild type and heterozygous animals responded normally compared to the untreated control" (col. 20, lines 24-26). Further statements in col. 20 make it evident that heterozygotes behaved identically to wild type when tested for various indicators of estrogen responsiveness. While Applicants recognize that each application

is treated on its own merits, Applicants submit that this patent, which carries a presumption of validity, stands as persuasive evidence that the Examiner is incorrect in maintaining that the written description requirement is not met in the instant case.

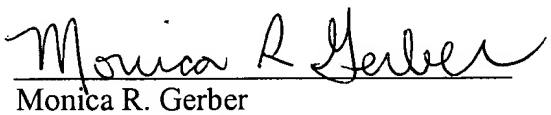
In summary, characteristics descriptive of the claimed homozygous, heterozygous and chimeric mice and of heterozygous cells have been provided in the specification. Such characteristics relate to the reduced expression of Erk5 in heterozygous and chimeric mice and heterozygous cells, to the reduced ability of heterozygous mice (and heterozygous offspring of chimeric mice) to give rise to viable offspring, and to the specific visible phenotype of the homozygous embryos that results when heterozygous mice (and heterozygous offspring of chimeric mice) are interbred. While Applicants submit that the written description requirement does not mandate that the description be contained in the claims themselves, the claims have been amended to specifically recite characteristics of the chimeric and heterozygous mice and of the heterozygous cells. Withdrawal of the rejection is respectfully requested.

In conclusion, in view of the amendments and remarks presented herein, the application and pending claims comply with the requirements of 35 U.S.C. §112. Applicants therefore respectfully submit that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

If, at any time, it appears that a phone discussion would be helpful in resolving any remaining issues, the undersigned would greatly appreciate the opportunity to discuss such issues at the Examiner's convenience. The undersigned can be contacted at (617) 248-5000 or (617) 248-5071 (direct dial).

Please charge any fees associated with this filing, or apply any credits, to our Deposit Account No. 03-1721. In addition, in accordance with 37 C.F.R. 1.136(a)(3), please consider this authorization to charge our Deposit Account to encompass any necessary Petitions for Extension of Time and fees associated therewith in the instant case.

Respectfully submitted,



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